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Studies of kidney cells in culture

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Research using kidney cells in culture has expanded from descriptive studies of the properties of cultures to exploration of mechanisms at the cellular and molecular level. The material available for study has increased as several primary cultures and new cell lines that have interesting differentiated properties have been developed. More options are available to promote cell growth and differentiation. Some of the ways cultures have been used are summarized in this review. Cell biologists have used cultured epithelia to advance understanding of the mechanisms by which epithelial form and maintain polarization, a property fundamental to epithelial function. Transport physiologists have used cultured epithelia in a variety of ways. Some studied cultured epithelia as they might have studied a naturally occurring epithelium. Others have taken advantage of specific properties of cultured cells that make it easier to apply standard techniques. A few have taken advantage of the fact that cultured material can be manipulated in many ways. Because of limitations of space and my own interest, only a few studies are described in detail. Throughout, I speculate on new directions for research with cultured kidney cells. The major challenge is no longer to grow cells that express differentiated function, but to study those functions with methods that are particularly productive because the cells are in culture.

Epithelial cell biology

Polarity

Cultured epithelia have been used to study the genesis and maintenance of epithelial polarity. The observation that certain enveloped viruses bud from infected epithelial cells in a polar fashion (each virus buds only from the apical or basolateral plasma membrane) [1] initiated a series of studies of polarity in epithelial cells [2]. Most of these studies have been performed with MDCK cells (Table 1). The glycoproteins of the viral envelope are inserted into the apical or basolateral plasma membrane of the infected epithelial cell before the polar budding of the virus [11]. Therefore the envelope glycoproteins of the viruses have been studied as model membrane proteins that are synthesized and then distributed and inserted in the plasma membrane in a polar fashion, perhaps in the same way as an apical membrane sodium channel or a basolateral membrane receptor for a peptide hormone. Since the viral glycoproteins can be detected using immunofluorescence or immunoelectron-microscopy, their synthesis, processing, and sorting can be followed. After synthesis in the endoplasmic reticulum, viral glycoproteins destined for the apical and those destined for the basolateral plasma membrane are found in the same golgi cisternae [12]. Sorting to the apical or basolateral plasma membrane follows processing in the golgi apparatus. The final glycoprotein retains information for polar sorting. When a viral glycoprotein that is normally inserted into the basolateral plasma membrane is experimentally fused into the apical plasma membrane it is sorted to the basolateral plasma membrane [13]. The genes for some of the viral glycoproteins have been cloned, sequenced, and expressed in transfected cells [14]. Experiments employing in vitro mutagenesis revealed that for both the hemagglutinin of influenza virus (an apical membrane glycoprotein) and the G protein of vesicular stomatitis virus (a basolateral membrane glycoprotein), the carboxy terminal end of the protein anchors the glycoprotein in the cell membrane. If that segment of the gene is deleted, the glycoprotein is secreted [15, 16]. Other mutations in the cytoplasmic domain at the carboxy terminal end affected different stages of intracellular transport of the influenza virus hemagglutinin [17]. Similar experiments transfecting epithelial cells may reveal the genetic signal for sorting and insertion into the apical or basolateral plasma membrane.

The polar budding of enveloped viruses from cultured epithelial cells has also been used to establish the polar distribution of phospholipids in the plasma membranes of epithelial cells [18]. When enveloped viruses bud, the envelope is formed from the lipid bilayer of the host cell and the viral glycoproteins that have been inserted in the plasma membrane. Thus, the lipids in the virus envelope are a sample of those in the plasma membrane through which the virus budded. When viruses bud from non-polar cells, all viral envelopes have the same phospholipid composition. When viruses bud from epithelial cells, the phospholipids in the envelopes of viruses that bud apically are different from those that bud basally. These observations confirm previous suggestions, based on analyses of membranes separated less completely, that the lipid composition of apical and basolateral plasma membranes are different.

Glycolipids are also distributed in a polar fashion in cultured epithelia. Monoclonal antibodies against an external site on a glycolipid in the apical plasma membrane of A6 epithelia do not react with an external site on the basolateral plasma membrane (Turner, Thompson, Sariban–Sohraby, and Handler, unpublished observations). Since lipids diffuse freely in the plane of

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Table 1. Continuous cell lines

Cell line	Origin	Reference
A6	Xenopus laevis kidney	3
CACO 2	Human colon carcinoma	4
GRB-MAL	Rabbit medullary thick ascending limb	5
HT-29	Human colon carcinoma	6
LLC-PK1	Pig kidney	7
MDCK	Dog kidney	8
OK	Opossum kidney	9
TB-6c	Bufo marinus urinary bladder	10

the plasma membrane, this implies that there is a barrier to diffusion of the glycolipid antigen in the region of the tight junction (the morphological boundary between apical and basolateral plasma membranes). Similar conclusions have been drawn based on the movement of fluorescent lipids incorporated into the plasma membrane of cultured epithelia. Those fluorescent lipids that did not flip–flop rapidly from one leaflet of the bilayer to the other did not move past the region of the tight junction [19]. Glycolipids, such as the antigen for the monoclonal antibody in the apical membrane of A6 epithelia, are known not to flip–flop from one leaflet of the bilayer to the other. These observations have been extended in studies of another glycolipid, the ganglioside GM1. Ganglioside GM1 is the specific high affinity membrane receptor for cholera toxin. The amount of GM1 in a membrane can be quantified by measuring the specific binding of radiolabelled cholera toxin. When the ganglioside GM1 content of the apical plasma membrane of A6 epithelia was increased by adding the ganglioside or the enzyme sialidase to the apical solution, the GM1 content of the apical plasma membrane increased tenfold. There was, however, no change in the GM1 content of the basolateral plasma membrane (Spiegel, Fishman, Blumenthal, and Handler, unpublished observations). Thus, several approaches lead to the conclusion that in the region of the tight junction, there is a barrier to diffusion of lipids within the outer leaflet of the plasma membrane. The nature of the barrier remains to be determined.

Since the tight junction is located at the anatomical junction of apical and basolateral plasma membranes, it has been imputed to have a role in maintaining polarity. If it has a role, it is probably not major in that enveloped viruses bud in a polar fashion from recently seeded epithelial cells before complete tight junctions have formed [20]. Attachment of epithelial cells is itself a manifestation of polarity, since that is a function of the basal plasma membrane. One other characteristic was noted in single epithelial cells within seven hours of attachment. Underlying the periphery of the cell is a filamentous ring that in mature epithelia has been shown to be composed of microfilaments. Perhaps the formation of the ring of microfilaments is an early and important step in the formation of a polarized epithelial cell [20].

Antibodies to membrane constituents

Antibodies have been used to select cells for culture, to follow the movement of specific antigens in epithelia, and to identify markers of specific cell types, an indication of differentiation. Monoclonal antibodies raised against MDCK cells were found to cross react with cortical collecting duct cells of dog kidney [21]. Dishes coated with the monoclonal antibody were then used to bind and thereby select cortical collecting duct cells from a suspension of cells prepared from collagenase–treated dog kidney cortex. Non-collecting duct cells were easily rinsed away from the antibody coated dishes. The technique yields a large number (10^7) of cells for primary culture [21, 22]. The primary cultures were polarized and, when grown on filters, had a transepithelial potential difference of 1.0 mV, apical surface negative. The cultures responded to bradykinin, vasopressin, and PGE_2 in an interesting way. Bradykinin stimulated PGE_2 release only when added to the apical surface. Vasopressin increased cAMP levels only when added to the basal side, but stimulated PGE_2 production when added to either the apical or basal side. An important observation was that on culture dishes, DD-AVP stimulated PGE_2 production as well as AVP, indicating that this is a response to a V_2 type (adenylate cyclase coupled) receptor [23]. It would be interesting to know whether the response to vasopressin added to the apical surface of epithelia on filters is also mediated by a V_2 type receptor, which in that case is not coupled to adenylate cyclase.

Polyclonal antibodies against leucine aminopeptidase (LAP) in the apical plasma membrane of MDCK cells were used to follow the movement of LAP after the antibody bound to LAP in the membrane [24]. Bound antibody was endocytosed and then reappeared in the apical plasma membrane. After endocytosis, LAP from an intracellular store followed by LAP with bound antibody were inserted in the apical plasma membrane in the region near the tight junction. This is not the region of insertion of all apical membrane proteins for the hemagglutinin of influenza virus (vide supra) is inserted uniformly over the apical plasma membrane [25]. Monoclonal antibodies to externally oriented apical membrane antigens of A6 epithelia were also endocytosed after binding to the apical membrane. Antibody to different antigens behaved differently. All were endocytosed but not all recycled to the apical plasma membrane. In addition, bound antibody interfered with normal polar sorting [26]. Can these antibody probes of membrane constituents be used to study the mechanism and signals of membrane turnover and sorting? Antibodies have been raised against purified transporters and used to follow the transporter (subunits of Na,K-ATPase) during its synthesis and insertion in the plasma membrane [27] and to clone the gene for the transporter (Band 3 of erythrocytes) [28]. Can useful antibodies be raised against transporters or other interesting membrane constituents that have not been purified?

Tamm–Horsfall protein is a membrane glycoprotein synthesized by cells of the thick ascending limb [29]. The expression of Tamm–Horsfall protein by a continuous line of cells derived from microdissected segments of rabbit medullary thick ascending limbs was demonstrated by immunofluorescence microscopy and used as confirmatory evidence for the origin of the cell line and as an indicator of differentiation [5]. Similar tactics have been used in studies of cultured glomerular cells. These studies and other aspects of glomerular cell culture have been reviewed recently [30–32]. Perhaps anti-Tamm–Horsfall protein antibodies or antibodies specific for other cell types could be used to select specific cells for culture, as the monoclonal antibody to MDCK cells was used to select canine cortical collecting duct cells. That strategy has been reviewed [22].

Growth and differentiation

Considerable effort has been expended to learn how to increase the growth and differentiation of cultured cells. There are many reports of primary cultures that express some differentiated properties of the renal cells of origin. These preparations have been used to study the factors that influence their growth and differentiation [33–35], but with few exceptions [36, 37], are difficult to initiate and do not yield large amounts of cell material. It is unlikely that such cultures will be very useful unless the cells can be persuaded to continue to grow and differentiate when subcultured. This is a major problem in the field. The continuous cell lines (for example, -MDCK, LLC-PK1, A6, TB-6c) that are most commonly used to study differentiated functions transformed (continued to grow) spontaneously. Unfortunately, the techniques (such as, -viral transformation, chemical transformation) that lead to immortality in culture, usually lead to loss of differentiation. There may be a fundamental incompatibility between continued growth and expression of differentiated properties. Most continuous epithelial cell lines that express differentiated functions do not do so until cultures are confluent and growth is arrested or markedly slowed (vide infra). Regulation of cell growth, a field of obvious importance, is beyond the scope of this review.

A variety of factors influence differentiation in culture. The culture medium is obviously important. Primary cultures of epithelial cells usually do best in medium with little or no serum, in part because the absence of serum discourages the growth of non-epithelial cells, in part because the long–term growth of epithelial cells is enhanced. A number of defined media (media in which specific hormones and growth factors replace serum) have been developed for specific primary cultures and continuous cell lines. A defined medium developed for LLC-PK1L cells, a strain derived from LLC-PK1 cells, has dramatic effects on the expression of receptors for vasopressin [38]. The strain can grow in the complete absence of serum, hormones, or growth factors, but under those conditions expresses only 5% of the adenylate cyclase response to vasopressin that is expressed by the parent strain grown with serum [39]. Even in medium with serum, the strain expresses only 15% of the response of the parent strain. In the defined medium, the strain expresses the full responsiveness of the parent strain [38]. The use of defined media eliminates phenotypic changes secondary to different lots of serum. In our laboratory we screen and select sera for culture of A6 cells, which form an epithelium that transport sodium actively (vide infra). Even though each lot of serum is pooled from many animals, there are large differences in sodium transport, epithelial resistance, and responsiveness to hormones among A6 epithelia grown with different lots of serum. A useful extension of this is the discovery of one lot of serum that, like other lots, supports cell growth and formation of an epithelium: unlike other A6 epithelia, those grown with this lot do not transport sodium in the absence of hormones (Paccolat, M. P., K. Geering, H. P. Gaeggeler, and B. Rossier, personal communication). Defined media have been reviewed recently [40].

In some continuous cultures routinely grown with medium containing serum, simple manipulation of the medium results in striking changes in differentiation. Cells of the continuous line designated HT-29, derived from a colon carcinoma, do not differentiate in standard media containing glucose. In medium without glucose or with glucose plus sodium butyrate, HT-29 cells form an epithelial monolayer with several differentiated characteristics of small intestinal epithelia. A potentially important discovery is that undifferentiated HT-29 cells have high levels of UDP-N-acetylhexosamines, whereas differentiated HT-29 cells have low levels. UDP-N-acetylhexosamine levels are low as well in CACO-2 cells which are also derived from a human colon carcinoma, but always differentiate on reaching confluency in normal medium containing glucose [41]. UDP-N-acetylhexosamines are intermediates in protein and lipid glycosylation. Are glycoproteins or glycolipids critical determinants of differentiation in these cells [41]? The addition of dexamethasone to the culture medium dramatically stimulates the differentiation of two epithelial cell lines [42, 43]. Does dexamethasone alter UDP-N-acetylhexosamine metabolism in these cells?

Differentiation is also influenced by the substrate upon which cells are grown. One approach has been to prepare a growth surface that resembles the normal extracellular matrix of the cells in situ. Increased differentiation and in some cases increased growth has been demonstrated in cultures grown on plastic tissue culture dishes coated with an extracellular matrix laid down by another culture [44]. The matrix forming cells may not be the same type of cell as those benefitting from it's presence. Alternatively, the extracellular matrix has been extracted from whole organs and added to culture dishes before cells from that organ are seeded [45]. Epithelia present a special problem since they grow, as in situ, with the basal plasma membrane attached to the culture dish. The basal plasma membrane is the plasma membrane that normally takes up nutrients and has receptors for growth signals. As cultures grow and differentiate, medium added above the cultures, as it is on dishes, may be excluded from the basolateral surface by competent tight junctions. Several preparations avoid this problem. Floating collagen gels are prepared by seeding cells on collagen gels attached to plastic culture dishes. After the culture is confluent, the gel is mechanically released from the dish and floats in the medium. Often, in the first few hours after release, the gel is contracted by the cells as they change from a flattened squamous epithelium to a cuboidal epithelium. For some cultures, the change in shape is also important. For example, primary cultures of mammary epithelial cells differentiate more on contracted floating gels than on non-contracted floating gels, which in turn manifest more differentiation than cells cultured on collagen gels attached to plastic dishes [46]. A variety of non-contracting porous surfaces have been used, including gels of collagen [47], filters made of mixed cellulose esters (Millipore) [48], and collagen coated polycarbonate filters (Nucleopore) [49]. Cells can be grown on filters lying on the bottom of a tissue culture dish or can be attached to a ring of plastic, forming a cup with the porous growth surface forming the bottom of the cup. The cups can be arranged so that separate solutions bathe the basal and apical surfaces. The latter arrangement has proven useful for facile measurement of electrical transport characteristics (such as short–circuit current and resistance [50]). A naturally formed growth surface, placental amnion, denuded of its own epithelial cells, supports the growth and differentiation of a cell line derived from rabbit

medullary thick ascending limb better than the forementioned synthetic supports [5].

The attachment surface can play a subtle role in differentiation. In early passages, glomerular mesangial cells are spindle–shaped and contract in response to vasopressin. In later passages they are more strap–like and, although they do manifest the early steps in the response, no longer change shape in response to vasopressin. To test whether the loss of the contraction response was the result of dedifferentiation or increased cell adherence to the culture dish, late passage mesangial cells were grown on dishes treated with polyhydroxyethylmethacrylate, which impairs cell spreading and attachment. The late passage cells on polyhydroxymethylmethacrylate treated dishes were spindle–shaped and contracted in response to vasopressin. It appears that late passage cells attach more firmly to the culture dish than early passage cells, and that the firm attachment impairs shape change. Since contraction is evaluated as change in cell shape, agents that influence cell attachment can appear to affect contraction. These and other studies of cultured glomerular mesangial cells were reviewed recently [31].

Epithelial transport and hormonal regulation.

For a variety of reasons, cultured epithelia have been more suitable preparations for applying standard electrophysiological techniques than naturally occurring epithelia. For example, some naturally occurring epithelia have a dense glycocalyx over the plasma membrane. The glycocalyx makes it difficult to apply a patch clamp electrode and obtain the extremely high resistance seal the technique requires. Most cultured epithelia do not have a dense glycocalyx, and a number of cultured epithelial cells have been studied with the patch clamp technique. As in other patch clamp studies, the cellular function of some of the channels found remains to be determined. There are preliminary reports of two channels of interest, the amiloride inhibitable sodium channel of A6 epithelia [51], and a potassium channel blocked by barium in a continuous cell line, GRB-MAL1, derived from the medullary thick ascending limb of rabbit kidney [52]. The latter studies illustrate an important feature of cultured material. Cultures may express some, but not all differentiated properties of the original tissue. Those properties that are present can be studied effectively. GRB-MAL1 epithelia do not have a transepithelial potential difference. Nonetheless, as in the tubule of origin, the apical membrane is hyperpolarized by furosemide and by barium, the latter reflecting the effect of barium on calcium activated potassium channels studied in detail by patch clamp [52].

Important information about the amiloride inhibitable sodium channel was obtained using epithelia formed in culture by A6 cells, a continuous cell line derived from the kidney of *Xenopus laevis* [3]. When grown on porous supports such as millipore filters, A6 epithelia transport sodium actively from apical to basal surface [53]. Sodium transport is inhibited by low concentrations of amiloride [53, 54], and by activators of protein kinase C, such as phorbol esters [55]. It is stimulated over a period of hours by aldosterone [53, 54] and more rapidly by hormones such as vasopressin [50], adenosine [56], PGE [57], and isoproterenol [57], which stimulate adenylate cyclase activity. Sodium transport was characterized in the intact epithelium by studying the kinetics of ^{22}Na uptake and the inhibition of uptake by amiloride. The $k_{1/2}$ for inhibition of sodium uptake by amiloride was 70nM [54]. Similar kinetics were found for amiloride inhibitable sodium uptake in apical membrane vesicles prepared from A6 epithelia [58]. To study the properties of single channels, vesicles were incorporated into thin lipid membranes [59]. Although A6 cells were used in the foregoing studies, there is no a priori reason for using material from a cultured rather than a naturally occurring epithelium.

Another example of fruitful studies of cultured epithelia that might have been performed using naturally occurring epithelia are those that proposed the concept that aldosterone acts by inducing the methylation of lipids and/or proteins. Epithelia formed by TB-6c cells, a continuous cell line derived from toad urinary bladder [10], were used. Aldosterone stimulates sodium transport in TB-6c epithelia in a fashion resembling that seen in toad urinary bladder and A6 epithelia [60]. The stimulation of sodium transport by aldosterone was associated with increased methylation of whole cell lipids and proteins, assessed by the incorporation of ^{3}H-methyl groups from exogenous [methyl-^{3}H]methionine. When methylation was blocked by the inhibitor 3-deazaadenosine (DZA), the stimulation of sodium transport was also blocked [61]. The foregoing studies with Tb-6c cells were highly suggestive of a role for methylation in the action of aldosterone. They were not conclusive because methylation reactions are so common and vital in the life of a cell. At this point, an important difference between cultured material and naturally occurring material became critical. In contrast to apical membrane vesicles prepared from toad urinary bladder, in which no effect of aldosterone on amiloride inhibitable sodium fluxes could be detected even through sodium transport had been stimulated in the bladder [62], amiloride inhibitable sodium flux was elevated in apical membrane vesicles prepared from aldosterone treated A6 epithelia [58]. The retention by A6 vesicles of the effect of aldosterone permitted exploration of the role of methylation reactions directly on apical membranes. Vesicles prepared from control A6 epithelia were incubated with the methyl donor S-adenosylmethionine (adomet) for 12 min. Amiloride inhibitable sodium flux rates increased to those of vesicles prepared from aldosterone treated epithelia. In contrast, adomet had no effect on vesicles prepared from aldosterone treated epithelia. Tracer studies with the vesicle preparation showed that incubation with S-adenosyl-[^{3}H-methyl]methionine increased methylation of lipids and proteins. As anticipated, all the effects of adomet were blocked by DZA [63]. Several aspects of these important studies warrant further exploration. For example, when vesicles from aldosterone stimulated epithelia were incubated with DZA, there was a reduction in amiloride inhibitable sodium flux to levels seen in control vesicles [63]. If DZA acts solely by inhibiting methylation reactions, the last observation indicates that in vesicles prepared from aldosterone treated epithelia, there must be rapid turnover of methylated substrates, in turn implying that methyl donors and methylating and demethylating enzymes are present in the vesicle preparation. It may be possible to establish this and determine which part of the cycle is different in the preparation from aldosterone treated epithelia. Is the difference the aldosterone induced protein that simulates sodium transport? Can the vesicles be purified enough to allow identification of the membrane constituent that is the substrate for methylation? Can the vesicle preparation be used to study the action of

cAMP (or protein kinase A) or of phorbol esters (or protein kinase C)?

Cultured epithelia are particularly suitable for studying the effects of prolonged exposure to an agent or a particular set of conditions. This is not so easily achieved in naturally occurring tissues in situ because of the influence of other cells and regulatory mechanisms. Most isolated epithelia cannot be maintained in vitro for more than 24 hr. Epithelia formed by LLC-PK1 cells, a continuous cell line derived from the kidney of a hampshire pig [7], express several differentiated properties of interest to renal physiologists. Upon reaching confluence, they express an apical membrane sodium–coupled hexose transporter like that in the pars recta of the proximal tubule. The transporter has been identified in a number of ways which illustrate the application of a variety of standard techniques for studying transport. The transporter was first identified by demonstrating sodium dependent uptake of alpha–methyl–glucoside (AMG) into epithelia grown on collagen coated nucleopore filters [64, 65]. Subsequently it was shown that the addition of glucose to the apical solution of LLC-PK1 epithelia grown on filters resulted in stimulation of short–circuit current, a reflection of increased sodium transport coupled to glucose transport [66]. Net transport of glucose was also demonstrated in epithelia grown on filters [67]. Finally, apical membrane vesicles from LLC-PK1 epithelia manifest sodium–coupled hexose transport [68–70]. In all of the foregoing preparations, the coupling ratio was found to be 2:1, sodium:hexose, as described for the pars recta. Continuous cell lines are usually subcultured weekly. However, when the cultures were maintained on the same plastic tissue culture dish for as long as three weeks, the expression of the sodium–coupled hexose transporter increased with time in a linear fashion. This was found in studies in which transport was assessed by measuring the steady–state accumulation of AMG in epithelia grown on plastic tissue culture dishes compared to its concentration (~0.1 mM) in the medium [69]. Steady–state accumulation increases as uptake increases and as leak out of cells decreases. Since the latter did not change under any of the experimental conditions, changes in steady–state accumulation reflect changes in uptake [71]. This was confirmed by the finding that sodium–coupled AMG flux rates were increased in apical membrane vesicles prepared from LLC-PK1 epithelia cultured for longer periods.

A series of studies demonstrated that in LLC-PK1 epithelia, the number of sodium–coupled hexose transporters is regulated by the growth medium concentration of glucose, the substrate for the transporter. Raising the concentration of glucose in the culture medium to 25 mM resulted in epithelia which had lower levels of AMG accumulation and fewer specific binding sites for phlorizin than epithelia grown in medium containing 5 mM glucose. The response was specific for the transporter in that there was no change in the activity of alkaline phosphatase, an enzyme considered to be a marker of the apical membrane [71]. The epithelia were responding to a metabolic signal rather than interaction of hexose with the sodium–coupled transporter. The addition of AMG (which is transported but not metabolized) to the culture medium had no effect on the number of transporters. LLC-PK1 cells consume glucose very rapidly, so that when fed medium containing 5 mM glucose, the concentration of glucose in the medium was less than 0.5 mM more than half the time. Other hexoses such as fructose and mannose, which do not interact with the transporter but are metabolized, albeit more slowly than glucose, had an effect like that of glucose although not as marked [72]. The intracellular metabolic signal remains to be identified. The transport response to a shift in the concentration of glucose in the medium is not detectablea for 48 hr [71]. The slow response raised the question of whether cell division is required for expression of the response. This was ruled out in studies in which cell division was inhibited by high doses of gamma irradiation. The response was still evident in cells in which the incorporation of thymidine into DNA was eliminated by irradiation [73].

LLC-PK1 epithelia also express sodium–coupled phosphate transport, demonstrated as saturable sodium–coupled phosphate uptake by intact epithelia [74, 75]. Since phosphate uptake by intact cells may include a component of incorporation of phosphate into metabolic pools, the apical membrane transporter was studied more directly in apical membrane vesicles [76]. The transporter resembles the sodium–coupled phosphate transporter in the proximal tubule. In LLC-PK1 apical membrane vesicles, the Km for phosphate was 99 μM at physiologic concentrations of extracellular sodium, and arsenate inhibited phosphate transport competitively. Two sodium ions moved with one phosphate ion. Transport was unaffected by a potential gradient across the vesicle membrane, indicating that the transport was probably electroneutral. Like the sodium–coupled hexose transporter, the sodium–coupled phosphate transporter is regulated by the concentration of its substrate in the growth medium [77]. In contrast to the hexose transporter, the response of the phosphate transporter is very rapid. Phosphate deprivation for 10 min leads to an increase of 30% in the activity of the transporter. The response was also observed in apical membrane vesicles prepared from adapted cells, and was characterized as an increase in V_{max} without any change in Km [78]. Phosphate transport in LLC-PK1 epithelia was not affected by parathyroid hormone or cAMP. Another cell line is responsive. OK cells, a continuous line derived from the kidney of an opossum [9], also form an epithelium that transports phosphate in a sodium–coupled fashion, with a Km similar to that in LLC-PK1 cells. In OK epithelia, phosphate transport is inhibited by parathyroid hormone, acting via cAMP. Here too, the effect was evident in apical membrane vesicles prepared from epithelia exposed to parathyroid hormone, and was characterized as a reduction in V_{max} (Malmstrom and Murer, personal communication).

Other transporters of the proximal tubule have been identified in continuous cell lines. The Na^+/H^+ exchanger is expressed in LLC-PK1 epithelia and in apical membrane vesicles prepared from LLC-PK1 epithelia [79], and in OK epithelia [80]. In OK epithelia the transporter is regulated rapidly by PTH via cAMP [80] in a fashion resembling that in the proximal tubule.

The homogeneity of cell cultures is useful for studies of hormone interaction with receptors and receptor coupling to steps that transduce the hormonal signal to an intracellular signal. The interaction of vasopressin and its receptor coupled to adenylate cyclase has been studied very effectively in LLC-PK1 cells [81–83]. The recent discovery that atrial natriuretic factor raises cGMP levels in cultured renal papillary collecting duct cells [84], in LLC-PK1 cells (Goligorsky, M., S. Westbrook, P. Reinach, and K. Hruska, and Cantiello, P., and

D. Ausiello, personal communications) and in primary cultures of proximal tubule cells (Goligorsky et al, personal communication), and inhibits sodium transport (Goligorsky, Cantiello and Ausiello, personal communications) should lead to rapid advances in our understanding of the epithelial cell response that contributes to the natriuresis elicited by atrial natriuretic factor.

New approaches

Now that cultures are available that express interesting renal functions, how can they be used to advance our understanding of those functions? One approach might be to develop mutant cell lines and apply the techniques of somatic cell genetics. These techniques have been enormously powerful in dissecting the hormone regulated adenylate cyclase complex and understanding genetic abnormalities of adenylate cyclase in humans [85]. Mutants are usually selected by their ability to survive adverse conditions, as in two reports of MDCK cell mutants. In one, ouabain resistant mutant MDCK cells were selected by their ability to survive and grow in medium containing concentrations of ouabain that were lethal to the wild type. It was shown that 50% of the Na,K-ATPase in the mutants had a higher K_d for ouabain than normal, while 50% remained wild type for ouabain binding. There was no change in the rate of synthesis of the enzyme [86]. A similar protocol was used to select mutants of MDCK cells that could survive and grow in low potassium (<0.2 mM) medium. The mutants were shown to survive because they leaked potassium via a bumetanide–sensitive K,Na,2Cl cotransporter more slowly than the wild type [87]. Similar protocols had been used to select for mutants in non-polarized cells. More devious selection strategies may be required to select for other transport mutants. The technique of tritium suicide was used to select hexose carrier mutants in CHO cells, a fibroblastic cell line. Tritium suicide is achieved by loading wild type cells with tritium and then storing the cells in liquid nitrogen until radiation has killed cells containing tritium. Mutagenized CHO cells were incubated with 2-deoxy [^{3}H] glucose. Mutants defective in the hexose carrier did not take up the 2-deoxy [^{3}H] glucose and grew out after storage [88]. A similar protocol has been proposed to produce mutants in the sodium–coupled hexose transporter of LLC-PK1 cells [89]. A related strategy, H^+ suicide, has been used to produce mutants in the Na^+/H^+ exchanger in fibroblasts [90].

There are interesting strain differences in some common kidney derived cultures. For example, LLC-PK1 cells have many transport characteristics of the proximal tubule but like most cell lines are incapable of gluconeogenesis [91], a function of the proximal tubule. LLC-PK1 cells lack fructose-1,6-bisphosphatase activity [92]. A strain of LLC-PK1 cells has been isolated that expresses fructose-1,6-bisphosphatase activity and is capable of gluconeogenesis [93]. The two strains should be useful to evaluate the relationship of gluconeogenesis to other functions such as ammoniagenesis and transport. There are at least two strains of MDCK cells. The strain available from American Type Culture Collection develops epithelia with a low transepithelial resistance (~100 ohm-cm^2) [47]. Another strain, available in Europe, develops epithelia with a much higher resistance (~4000 ohm-cm^2) [94]. There are other differences between the two strains. Are the other differences genetically related to the difference in resistance?

Can we develop strategies to select for mutations specific for renal functions? Can we use them effectively? Experience has shown that a positive answer to the first question leads to a positive answer to the second, often in a completely unexpected way.

It is evident that with the continuing expansion of the number of renal cell lines and culture techniques, there is a great opportunity to apply a variety of approaches to further our understanding of renal function. Certainly, information will be gained by applying standard techniques that have been applied to renal tissue in situ and in vitro. The greatest opportunity for gaining new insights into renal cell function lies in applying techniques from other disciplines such as cell and molecular biology.

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